IDENTIFYING PEPTIDE LIGANDS OF TARGET PROTEINS WITH TARGET COMPLEMENTARY LIBRARY TECHNOLOGY (TCLT)

FIELD OF THE INVENTION

This invention relates to methods of screening for peptide ligands of target proteins.

BACKGROUND OF THE INVENTION

Rational drug designs are more and more dependent on identifying binding ligands which interacts with a target protein and the ability to predict their points of contact. Peptide library technology has become a very powerful tool in this regard. Large collections of peptides with randomized sequences are prepared and displayed on the surface of genetic packages, i.e., replicable entities such as cells or viruses, especially phage. Peptides having specific binding properties are subsequently selected by multiple rounds of panning (selection by binding affinity to an immobilized target) and amplification of the bound genetic package, such as phage (1). Phage display peptide libraries have been successfully screened to identify peptides that bind to cell surface or intracellular receptors, substrates or inhibitors of enzymes, and epitopes recognized by polyclonal and monoclonal antibodies (2-10).

SUMMARY OF THE INVENTION

This invention features an improved method of screening for peptide ligands of target proteins. This method is more powerful than the random peptide library technology in that it utilizes a predefined pool of peptides containing ligands for a selected target protein. The peptides in the pool exhibit complementary hydropathy to the target protein. Peptide ligands are selected based on their ability to bind to the target protein. This method is hereinafter referred to as Target Complementary Library Technology (TCLT).

TCLT can be used in all occasions when random peptide libraries are used to identify ligands for target proteins. By identifying peptide ligands which can influence the biological activities of their target proteins, TCLT can be used to discover peptides of therapeutic utility. In addition, peptide ligands are useful for studying the dynamic structure of their target proteins. and such information can be used for designing functional pharmacological small molecules.

Applications of TCLT include, but are not limited to, the following:

1) Identifying peptides which functionally mimic idiotypic antibodies. By mimicking the anti-idiotypic antibodies of the beta type (Ab2beta) (62), a peptide ligand can block the antigen-binding site of an antigen-specific antibody. By mimicking the anti-idiotypic antibodies of the gamma type (Ab2gamma) (62), a peptide ligand can prevent antigen-binding of an entire class of antibodies, such as IgG or IgE. Specifically, the framework 2 and framework 3 (FR2 and FR3) segments of both the heavy chain and light chain of a immunoglobulin molecular can serve as the target sequence for binding peptides which mimic the function of an Ab2gamma antibody. Each of the complementarity - determining - regions (CDR1, CDR2 and CDR3) can serve as the target sequence for AB2 beta-mimicking peptides. The target proteins include IgE, IgG, IgA, and specific antibodies such as anti-acetylcholine receptor antibodies. Peptide ligands of these target proteins can be used for the prevention and treatment of allergy, and autoimmune diseases including rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosis, and autoimmune nephritis.

2) Identifying peptides which mimic the function of a biological active molecule, such as a hormone, a cytokine, a growth factor, or a chemical compound. The target proteins include the cell surface receptors and intracellular receptors for these molecules, including, but not limited to, the receptors for insulin, human growth hormone, erythropoietin, thrombopoietin, estrogen, androgen, epidermal growth factor, interferons, interleukins, fibrinogen, endorphin, enkephalin, dopamine, nicotine, acetylcholine, angiotensin, and somatostatin.

3) Identifying peptides which mimic the binding domain of a receptor. By binding to the receptor's native ligand, the peptide ligands act as decoys to compete with the receptor, thereby preventing the biological effects induced by the interaction between the receptor and its native ligand. The target proteins in this case include the biological active peptides and proteins, which include, but are not limited to, interleukin-1, interleukin-2, bradykinin, platelet activating factors, and tumor necrosis factor (TNF).

4) Identifying peptides which mimic the receptor-binding domains of viral or bacterial proteins. Such peptides can block viral or bacterial infections by preventing the virus or bacteria from binding to its cellular receptors. In this case the target proteins can either be the known cellular receptors for viruses/bacteria, such as ICAM-1 and LDL receptor which are the major and minor receptors for human rhinoviruses (63, 64), or a viral or bacterial surface protein known to bind to corresponding cellular receptors.

5) Identifying peptide ligands for any protein. The peptide ligand can either be used for affinity purification of the target protein, or as a probe for the detection of the target protein *in vitro* and *in vivo*.

6) Identifying an unknown receptor for a known ligand, and an unknown ligand for a known receptor. In this case, the known ligand/receptor would serve as the target and the corresponding receptor/ligand can be identified by one of the following approaches. First, the complementary DNA fragment selected by TCLT can be used as a probe to screen another library which contains the potential receptor (23). Second, the complementary anti-sense peptide encoded by the complementary DNA fragment can serve as an immunogen to generate antibodies which interact with the potential receptor with specificity (24-37).

TCLT involves constructing (i) a peptide library containing a collection of complementary peptides of a target protein/peptide, wherein the complementary peptides in aggregation complement all, substantially all (preferably no less than 70%, more preferable no less than 80%, even more preferably no less than 90%), or a significant portion of the target protein/peptide; or (ii) a nucleic acid library containing a collection of nucleic acids encoding the aforesaid complementary peptides. In the latter case, the nucleic acid library is inserted into expression vectors and introduced into genetic packages such as phage particles and the complementary peptides are expressed on the surface of the genetic packages. Peptide ligands are selected from the library by panning against the target protein/peptide or the binding domain of the target protein/peptide. The target protein/peptide or its binding domain is preferably immobilized on a solid support. Genetic packages which bind to the immobilized target protein/peptide can be amplified and subject to several more rounds of panning. Complementary peptides with sufficient specific affinity to the target protein/peptide are selected at the end of this process, and their sequences can be uncovered from the genome of the selected genetic packages.

By "hydropathy character of an amino acid" is meant the hydrophobic or hydrophilic nature of the amino acid. Specifically, it is reflected by the hydropathy score of the amino acid in Tables 1-3 of the specification. The hydropathy scores of the twenty amino acids in Table 1-3 range from +4.5 to -4.5, and allow for the grouping of the twenty amino acids as hydrophobic (+4.5 to +1.8, Table 1), hydrophilic (-4.5 to -3.2, Table 2), and slightly hydrophilic (-0.4 to -1.6, Table 3) (Ref. 11). Hydrophobic amino acids in Table 1 (i.e., Ile, Val, Leu, Phe, Cys, Met, and Ala) complement hydrophilic amino acids in Table 2 (i.e., Arg, Lys, Asn, Asp, Gln, Glu, and His), and *vice versa*. Slightly hydrophilic amino

acids in Table 3 (i.e., Gly, Thr, Trp, Ser, Tyr, and Pro) complement each other. For example, each of Arg, Lys, Asn, Asp, Gln, Glu, and His is complementary to Ile; and each of Gly, Thr, Trp, Ser, Tyr, and Pro is complementary to Gly. In other words, each of Arg, Lys, Asn, Asp, Gln, Glu, and His is a complementary amino acid to Ile; and each of Gly, Thr, Trp, Ser, Tyr, and Pro is a complementary amino acid to Gly.

By "hydropathy profile of a peptide or protein" is meant its profile as defined by the hydropathic characters of amino acids in the peptide or protein.

By "complementary peptide" is meant a peptide containing a contiguous amino acid sequence (in the direction from the amino terminus to the carboxy terminus or in the direction from the carboxy terminus to the amino terminus) which complements the amino acid sequence of the target protein/peptide according to the rule set forth above. Any target protein/peptide has a plethora of complementary peptides because each amino acid in the target protein/peptide has a number of complementary amino acids.

Sub. B2

By "anti-sense peptide" is meant a peptide encoded by the anti-sense strand of the target gene translated in either 5' to 3' or 3' to 5' directions. For example, if the sense strand of the target gene has 5' CUU GUU CUU UUU 3' encoding a peptide Leu-Val-Leu-Phe, the anti-sense peptide would either be a peptide having the sequence Lys-Lys-Asn-Lys as encoded by the anti-sense strand 5' AAA AAG AAC AAG 3' or a peptide having the sequence Glu-Gln-Glu-Lys as encoded by 5'GAA CAA GAA AAA 3'. The matching pattern in Tables 1-3 shows that the anti-sense strand of a target gene usually encodes complementary peptide to the target protein/peptide. Anti-sense peptides which are complementary to the target protein/peptide are called complementary anti-sense peptides.

By "target complementary library (TCL)" is meant (i) a peptide library containing a collection of complementary peptides of a target protein/peptide, wherein the complementary peptides in aggregation complement all, substantially all (preferably no less than 70%, more preferable no less than 80%, even more preferably no less than 90%), or a significant portion of the target protein/peptide; or (ii) a nucleic acid library containing a collection of nucleic acids encoding the aforesaid complementary peptides. In a preferred embodiment, the complementary peptides are linked to flanking sequences.

By "anti-sense target complementary library (anti-sense TCL)" is meant a target complementary library wherein the complementary peptides are also anti-sense peptides of the target protein/peptide. These anti-sense peptides in combination complement the amino acid sequence of the target protein/peptide in its entirety or

substantial entirety (preferably no less than 70%, more preferable no less than 80%, even more preferably no less than 90%). In a preferred embodiment, the anti-sense TCL is a gene fragment library wherein fragments of the anti-sense strand of the target protein are operatively linked to a promoter for expressing the anti-sense peptides. The sizes of the nucleic acid fragments encoding anti-sense peptides may be defined or random. The anti-sense peptides encoded by the gene fragments may overlap each other randomly or in a predefined manner. In a further preferred embodiment, fragments of the anti-sense strand are linked to flanking sequences.

By "comprehensive target complementary library (cTCL)" is meant a target complementary library containing or encoding a collection of complementary peptides. Each complementary peptide of the collection complements the entire length of the target protein/peptide. In addition, this collection includes all possible complementary peptides to the target. In other words, each amino acid in the target is complemented by all its possible complementary amino acids at the corresponding position respectively in different complementary peptides of the collection. For example, the cTCL of the target peptide Ile-Gly-Arg would contain a collection of complementary peptides represented by the formula X-(Arg, Lys, Asn, Asp, Gln, Glu, or His)-(Gly, Thr, Trp, Ser, Tyr, or Pro)-(Ile, Val, Leu, Phe, Cys, Met, or Ala)-Y, wherein each position of the complementary peptides is fully randomized according to the formula and X and/or Y represent either nothing or one or more amino acid residues. This cTCL would have at least 7x6x7 = 294 different members.

By "target protein/peptide" is meant any protein/peptide or a domain within any protein/peptide.

By "ligand" is meant a peptide or protein capable of binding directly to the target protein/peptide.

By "binding domain" or "interaction domain" is meant a domain or region of a protein which is primarily responsible for the protein's ability to bind to another protein.

By "genetic packages" is meant replicable entities capable of carrying the nucleic acids of a target complementary library (TCL), including, but not limited to, vegetative bacterial cells, bacterial spores, bacterial viruses and eukaryotic cells. Genetic packages allow the amplification and recovery of the encapsulated genetic message following selection. A preferred genetic package is a phage. A phage display library expresses a collection of cloned proteins on the surface of phage particles as fusion with a phage coat protein. In connection with the genetic packages, "select" or "selection" means

enriching a subpopulation of genetic packages displaying a desired phenotypic characteristic (such as affinity to a probe).

Other features and advantages of the invention will be apparent from the detailed description of the invention below and from the list of enumerated embodiments that follows.

DETAILED DESCRIPTION OF THE INVENTION

The molecular recognition theory and complementary hydropathy

Without being bound by any theory, applicant's invention is supported by the molecular recognition theory (MRT) proposed by Blalock et al. in 1984 (11).

The molecular recognition theory arose from the observation that genetic codons for hydrophobic amino acids on the sense strand of a double-stranded DNA are complemented by genetic codons for hydrophilic amino acids on the anti-sense strand of the same ds DNA, and vice versa (11-13). The DNA sequences in which the amino acids encoded by one strand are hydropathically complemented by the amino acids encoded by the other strand are referred to as the complementary sequences. Two peptides encoded by complementary sequences in the same reading frame show a total interchange of their hydrophobic and hydrophilic amino acids when the amino terminus of one is aligned with the carboxy terminus of the other. The molecular recognition theory predicts that such inverted hydropathic pattern may allow pairs of complementary peptides to assume conformations that enable the pair to interact with each other specifically (11).

This hypothesis was initially tested in the binding of corticotropin (ACTH) to its complementary anti-sense peptide (HTCA). Indeed, the synthetic peptide HTCA bound to ACTH in a saturable, high affinity, specific fashion (14). Later on, specific interactions between pairs of complementary peptides were shown by more than forty different proteins (summarized in 15). High affinity anti-sense peptides have been used in affinity chromatography to purify a number of biologically active proteins (16-19).

It follows from the molecular recognition theory that antibodies against a complementary anti-sense peptide of a peptide ligand would bind to the receptor of this peptide ligand. In fact, scientists have used this approach to identify unknown receptors for known ligands, e.g., receptors for angiotensin II, fibronectin, vasopressin, β-endorphin, γ-endorphin, and enkephalin (24-37).

Recently, Martins et al. (36) identified the cellular receptor for human prion with an anti-sense peptide. They synthesized a complementary anti-sense peptide to the

neurotoxic region of the human prion (residues 106-126) and raised antibodies against this complementary anti-sense peptide. It turned out that these antibodies specifically stained the surface of neurons and recognized a 66-KD membrane protein which binds to prion (PrPc) both *in vitro* and *in vivo*. Furthermore, both the complementary anti-sense peptide and antiserum against it inhibit the toxicity of prion towards neuronal cells (36).

Consistent with the molecular recognition theory, it has been reported in many instances that the complementary sense peptide of a ligand pairs with the binding domain of its receptor in the same way it pairs with its complementary anti-sense peptide at the DNA level and the protein level (20-23). In that regard, DNA probes derived from the coding sequence of peptide ligands have been used successfully to identify cDNA clones of their receptors from a cDNA library (23).

These examples show that the amino acid sequence of a receptor can be deduced from the amino acid sequence of its ligand at the region of their interaction, and vice versa, because these two sequences complement each other hydropathically. It has been shown that a pair of idiotype and antiidiotype antibodies (Ab1 and Ab2) can be generated by using as immunogens a pair of complementary sense and anti-sense peptides.

In a frugal biological system developed under constant evolutionary pressure, a peptide or protein that does not interact with another peptide or protein is of little value to cells and organisms. The molecular recognition theory suggests that nature may have evolved a fail-safe system to ensure that this does not occur. Because of the complementarity of nucleic acids and the interactive qualities of the peptides encoded thereby, a strand of nucleic acid contains not only the information for one peptide but also that for a second peptide which is meant to interact with the first (38). It has been postulated that the interacting domains (encoded by the functional exons) of two proteins, such as a receptor and its ligand, co-evolved in this manner (38-40).

In human genes, short complementary sequences (about 15 AA in length on average) are frequently found at intervals of approximately fifty amino acids (15, 41). The majority of complementary sequences are amphophilic containing reverse turns, implying that these regions are on the molecular surface at the beginning or end of automatic folding units, helices and beta sheets. This phenomenon implies that these regions are important for folding and maintaining the structural integrity of proteins (15). Therefore, complementary anti-sense peptides which bind to a target protein would affect the biological activities of the target protein. Numerous examples have illustrated that

biologically active proteins such as antibodies, cytokines, and hormones can be functionally affected by the binding of complementary anti-sense peptides (42-56).

According to the molecular recognition theory, peptide ligands for any target protein could be identified among the complementary peptides of the target protein, numerous experimental observation also reveal the specific binding between peptides with opposite hydropathy profiles. Therefore, an efficient way of identifying ligands for a target is to screen a pool of complementary peptides.

Sequences of complementary peptides to a target protein/peptide can be constructed by using computer programs (e.g., *see* 15, 70). However, because the "native" hydropathy of a peptide is not only determined by its sequence, but also strongly influenced by the flanking regions, a better TCL is prepared by linking appropriate flanking amino acids to complementary peptides (15).

A relatively simple way of constructing a TCL is to prepare a gene fragment anti-sense TCL. In this method, the TCL is prepared by amplifying fragments of the target gene by polymerase chain reaction (PCR) using random hexamer oligonucleotide primers or other universal primers (57). Because sequences encoded by the anti-sense DNA strand of the target gene is just as well represented as that of the sense strand, a complete collection of the anti-sense peptides with their various flanking sequences are encoded by approximately one half of the genetic packages so prepared. Optionally, the fraction encoding the sense strand peptides could be excluded by subtractive hybridization following the generation of a single stranded nucleic acid (e.g., by transcription).

A cTCL can be synthesized as follows: residues with complementary hydropathy scores (as defined in Tables 1-3 and refs. 11-13) to each residue in the target sequence are randomly incorporated into the corresponding positions in the library. As a result, the TCL contains all the peptides which complement the target sequence hydropathically. This method is particularly suitable for screening for peptide ligands when the target is a short region in a protein of known sequence.

TCL can be displayed on phage as either fusion proteins with the minor phage coat protein pIII or the fusion proteins with the major phage coat protein pVIII. Three to five copies of pIII fusion proteins are expressed per phage particle, therefore they are used to display peptides with good binding affinities. Because pVIII fusion proteins are expressed at a much higher level at about 2,700 copies per particle, they can be used in a first screen when the binding affinities are suspected to be weak (1).

To select for peptides in the cTCL which have higher affinity to the target than the complementary anti-sense peptide, the complementary anti-sense peptide is synthesized and included in the elution buffer used to elute phage particles from the immobilized target protein/peptide. The concentration of the complementary anti-sense peptide determines the stringency of the elution buffer, the higher the concentration, the higher the stringency. Phage particles which withstand the high stringency elution carry peptides with high affinity to the target protein/peptide.

Once a binding peptide is identified through an initial screening, the sequence of this peptide is used as a guide for the generation of a dedicated library which contains various mutants of the initial binding peptide. Peptides with better binding affinity than the binding peptide from the initial screening will be selected by panning the dedicated library against the immobilized target protein. This procedure is described in detail in U.S. patent 5,223,409, incorporated by reference herein in its entirety.

Peptides with higher affinity can also be generated by linking a peptide identified by TCLT to the polymerization domain of a coiled-coil protein (58), such as GCN4(59) or COMP(60), to create a tetravalent or pentavalent binding molecule. An increase in affinity by 10^5-10^6 folds has been demonstrated with this approach (60).

Occasionally, high-affinity ligands could not be found from libraries of linear and unstructured peptides because a constrained peptide with the right conformation confers more thermodynamic advantage than a peptide which changes its shape constantly (61). This can be remedied by introducing some structural constraints such as disulfide bonds to the peptides, to enhance the chance of finding a high-affinity ligand. A constrained TCL can be prepared by carrying PCR reaction with cytosine-containing random hexamer oligonucleotide primers in which either cytosine is present at various positions in every hexamer oligonucleotide primers, or one additional cytosine is added at the 5'- or 3'- end of the random hexamer oligonucleotide sequences. Cystein can be synthesized at both ends of the synthetic library.

Example 1: Design and Construction of a Comprehensive Target Complementary Library (cTCL)

Sub. B3

~~Phage fUSE5~~ is used as the vector for the cTCL. The library is made by ligation of synthetic degenerate BglI deoxyoligonucleotides fragments into the Sfi I site of the fUSE5, and transfection of *E. coli* with the ligation products by electroporation. The fUSE5 vector and the cloning procedure is described in detail in ref. 71, 72 and 89. The

~~synthetic degenerate BglI deoxyoligonucleotides fragments are synthesized by solid-phase phosphoramidite chemistry (90), and carry the following sequence:~~

Sub B3

5'-CTGTCAGGGCCCGAGGGGCT(XXX)nGGGGCCGCTGCGGCCTGTCAGG-3' (SEQ ID NO. 1)

In the above sequence, n is the number of amino acid residues in the target peptide. The degenerate sequence of (XXX)n is designed by the following principle: hydrophobic amino acids which are complementary to the hydrophilic amino acids in the target peptide are randomly incorporated in the same positions in cTCL; hydrophilic amino acids which are complementary to the hydrophobic amino acids in the target peptide are randomly incorporated in the same positions in sTCL. Slightly hydrophilic amino acids are used in sTCL corresponding to similar residues in the target peptide. The hydrophilic and hydrophobic amino acid residues are defined by their hydropathic scores as given in Tables 1-3.

To synthesize XXX triplets for hydrophilic amino acids, the second base comprises a mixture containing A and G at molar ratio 6:1; the first base consists of equimolar mixture of A, G, and C. Thymine is not included in the mixture because its presence in the first position would give rise to the stop codon TAA or TAG (91). The third base includes only an equimolar mixture of G and C which is designed to favor codons used by *E. coli* to express its most abundant proteins (91).

To synthesize XXX triplets for hydrophobic amino acids, the second base comprises a mixture containing T and C at molar ratio 5:2; the first base consists of mixture containing an A:T:C:G molar ratios of 3:3:3:1. The third base again includes only an equimolar mixture of G and C.

To synthesize XXX triplets for slightly hydrophilic amino acids, the first base consists of an equimolar mixture of A, T, C and G; the second base contains a mixture of G, C, and A molar ration 2:2:1, and third base includes an equimolar mixture of G and C.

The oligonucleotides are purified by denaturing PAGE, their complementary strands are synthesized by Klenow DNA polymerase (91). They are subsequently digested with BglI and ligated with fUSE5 (71, 72, 89).

A cTCL carrying peptides with ten amino acid residues will contain approximately 2.8×10^8 independent clones, which can be completely amplified as a phage display library. A cTCL carrying peptides with eleven amino acids will have approximately 2×10^9 clones.

Example 2: Blocking antigen binding activity of an antibody with peptide ligands targeting the second framework segment (FR2) in the heavy and light chain variable regions (VH and VL)

Variable regions of both the heavy chain and light chain of an immunoglobulin molecule are composed of four framework segments (FR1, FR2, FR3 and FR4) and three complementarity-determining segments (CDR1, CDR2 and CDR3) with the three CDRs separating the four FRs. The residue numbers for these segments were defined by Kabat et al. (80). The CDRs are the segments with highly variable amino acid sequences and are primarily responsible for antigenic specificity of each antibody. The FRs are the relatively conserved segments which provides structural framework for the antigen-binding domain of an antibody. The FR2 of light chain constitutes amino acid 35 to 49, and FR2 of heavy chain constitutes amino acid 36 to 49 (80).

The antigen-binding site is formed by the convergence of six hyper-variable peptide loops which represent six CDRs of both heavy and light chains (81). The FR2 segments of each chain also directly contribute to an antigen-binding site. The FR2 segments of heavy and light chains constitutes the side and base of the antigen-binding surface formed by six CDRs, and appear to be an integral part of the site. Parts of the FR2 seem to be as exposed as the CDR3 loops. In addition, the closest contact points between VH and VL domains of Fab are between the FR2 of the heavy chain and the CDR3 of the light chain, and vice versa. Therefore, the FR2 plays an important role in keeping the structural integrity of Fab since VH and VL need to be closely apposed to each other to form a complete antigen-binding site. Consequently, a polypeptide (or a chemical compound) which specifically binds to the FR2 segment can disrupt the antigen-binding ability of an antibody molecule by (1) altering the topography of an antigen-binding site and thereby preventing antigen binding; and/or (2) acting as a structural hindrance to prevent close contact between the FR2 and the CDR3s in both chains and thereby disrupting the structural integrity of an antigen-binding site.

Because FR2 is the most conserved segment in the variable region, a peptide targeting FR2 may mask antigen-binding activity of antibodies regardless of their antigenic specificities. Furthermore, peptides targeting the FR2 segments in antibodies of different isotypes can be used to mask antigen-binding activities of all the antibodies of the same isotype, i.e., IgE, IgG, IgA, IgM and IgD.

An FR2 targeting peptide can be identified by the following approaches:

Synthesize a TCL based on the sequences of FR2 segments and the complementary hydropathy and select peptide ligands by their ability to bind to and disrupt the functions of antibodies with different antigenic specificities. The following examples illustrate how to identify anti-IgE peptides.

A very simplified version of this approach is to identify the FR2 complementary sequences by computer programs (15, 70). Such approach generally produce peptides with binding affinity within the range of 10^3-10^6 M^{-1}. The affinity can be improved by screening a constrained TCL which contains various flanking sequences and cysteins at both sides of the complementary peptides (61).

Example 3: Blocking Human Rhinovirus (HRV) Major Receptor (ICAM-1) with Peptide Ligands

Sub B4

Human rhinoviruses cause about 70% of common cold. ICAM-1 serves as the cellular receptor for majority of HRVs (82). The extracellular part of the ICAM-1 molecule is composed by five immunoglobulin-like domains (D1-D5). Mutational analysis of ICAM-1 has shown that domain D1 contains the primary binding site for rhinoviruses as well as the binding site for its natural ligand lymphocyte function-associated antigen 1 (LFA-1) (83-86). The regions in D1 which have been implicated as the contact sites with HRVs include residues 1, 2, 24-29, 40-49, and 70-77 (83-87). Accordingly, peptide ligands of ICAM-1 targeting these regions may prevent the binding of HRVs to ICAM-1. Suitable peptide targets include, but are not limited to the following:

Residues 1-5: QTSVS (SEQ ID NO. 2)

Residues 24-29: SCDQPK (SEQ ID NO. 3)

Residues 40-49: KELLLPGNNR (SEQ ID NO. 4)

Residues 70-77: PDGQSTAK (SEQ ID NO. 5)

Peptide ligands capable of binding to each of the above target peptides are identified by the following procedure:

(1) Preparing cTCL (or constrained cTCL) for each target peptide, and displaying the library on phage; (2) Immobilizing each target peptide on a solid surface (Alternatively, the D1-D2 part of ICAM-1 molecular is expressed and purified in vitro as described in ref 88, coated onto a solid surface, and used to screen cTCLs); (3) Selecting phage particles by panning as described in detail above; and (4) Recovering the peptide sequences from the selected phage particles.

Peptide ligands identified by screening cTCLs are synthesized and tested for their ability to prevent HRVs infection to Hela cells. The experimental protocols are described in US provisional patent application 60/067,119 filed December 1, 1997, incorporated by reference herein.

The affinity of the peptides can be improved by linking the peptides with the polymerization domain of a coiled-coil protein to create a multivalent binding molecule as described in detail in provisional patent application 60/067,119.

Example 4: Identifying anti-IgE peptides with TCLT

The most common form of allergic reaction is allergic rhinitis (nasal allergy) which affects about 20% of population (66). Allergic rhinitis can be either seasonal which indicates pollen-allergen sensitivity (also referred to as hay fever), or throughout the year (perennial rhinitis) which tend to be caused by house dust, dust mites, and animal danders (66). Allergic rhinitis is associated with irritating, sometimes disabling symptoms involving upper respiratory tract and eyes, it also imposes morbidity by leading to frequent secondary infections and even asthma attacks.

At cellular level, nasal allergy is caused by inflammatory mediators released by the mast cells, such as histamine, serotonin, prostaglandins, and various cytokines. Nasal allergy is also associated with basophils which have been presensitized by IgE.

IgE is the least component of the immunoglobulin family in human and animals. Its concentration in normal human serum is very low. Most IgE antibodies bind to the surface of mast cells and basophils through the Fc type 1 receptors (FcRI). Binding of IgE to the corresponding allergen causes clustering of IgE molecules which in turn activates mast cells and basophils and results in the release of inflammatory mediators.

Many attempts have been made to block the binding of IgE to FcRI as a way to prevent allergy. However, none of them have been successful so far in dissociating the preformed IgE-FcRI complex because the affinity between IgE and FcRI is as high as any good antibody-antigen interaction with the affinity constant (k_A) measuring in the range of 10^{-10} M^{-1} (67, 68). In addition, trying to prevent allergy by antibodies against other parts of the IgE molecule is almost bound to fail because an anti-IgE antibody can cause cross-linking of the IgE molecules on cell surface and thereby activate the mast cells and basophils and triggers an allergic reaction.

One approach of preventing allergy is to mask the antigen-binding site of IgE with a peptide which only binds to a single IgE molecule and does not cause cross-

linking of the IgE molecules. Such peptides target IgE at or near the antigen binding site and prevent antigen binding by causing steric hindrance to the binding site.

It would be preferable that the peptides target the conserved framework regions rather than the hypervariable regions in order to affect IgE molecules of a wide range of antigenic specificities.

TCLT is suitable for finding such peptides. First, it is well-established that the antigen binding site in an antibody molecule (Ab1) can be interfered by anti-idiotype antibodies (Ab2) against this antibody (Ab1). One type of anti-idiotype antibody, Ab2beta, is the direct mirror image of the antibody binding site on Ab1. Another type of anti-idiotype antibody, Ab2gamma, is directed against an idiotype near the binding site and causes steric hindrance to the antigen binding site (62). Second, idiotypes of an antibody are composed by peptide sequences found in both the hypervariable regions (CDRs) and the conserved framework regions of a antibody molecule (62, 69, 70). Therefore, a peptide targeting the conserved region of an idiotype can alter the idiotype and affect the antigen-binding ability of an entire class of antibody, such as IgE, IgG and IgA. Kang et al. have demonstrated the presence of complementary sequences in human immunoglobulin (52).

Peptides affecting antigen-binding ability of human IgE molecules can be identified with TCLT as described in the following examples.

Example 5: Blocking antigen-binding ability of an antibody molecule by targeting the framework 2 (FR2) region of the antibody

~~Based on their sequences, the heavy chain (H) of an antibody can be classified into six families~~ (V_H1 to V_H6). The general sequences of FR2 region in each family are in the following:

V_H1: W V R/Q Q A P/H/T G/A K/Q G/E/R/A L E/G W M/I G (SEQ ID NO. 6)

V_H2: W I R Q P P G K A L E W L A (SEQ ID NO. 7)

V_H3: W V/I R/H Q A P/Q G K G L/P E/V W/Y/L V S/A/G (SEQ ID NO. 8)

V_H4: W I/V R Q P P G K G L E W I G (SEQ ID NO. 9)

V_H5: W V R Q M P G K G/E L E W M G (SEQ ID NO. 10)

V_H6: W I R Q S P S R G L E W L G (SEQ ID NO. 11)

The sequence of the light chain (L) of an antibody is:

~~V_L kappa: W Y Q Q K P G Q/K P/S/A P K L L I Y (SEQ ID NO. 12)~~

Human IgE molecules are mostly composed of heavy chain genes from the V_H5, V_H3, V_H4 and V_H6 families (92,93), therefore the FR2 sequences from these families plus

the FR2 from the light chain serve as the target sequences for an IgE blocking polypeptide in this invention.

The IgE-blocking peptide can be designed directly based on the molecular recognition theory as in the following:

~~Peptide 1(binds to FR2 in V_H5): P D A L H G P F A Q(or D) L P H P (SEQ ID NO. 13)~~

~~Peptide 2(binds to FR2 in V_H3, V_H4 and V_H6): P D A L G/R G P F A Q/D L P N P (SEQ ID NO. 14)~~

~~Peptide 3(binds to FR2 in V_L kappa chain): P V L L F R P L R G F E E D I (SEQ ID NO. 15)~~

To isolate peptides with higher affinity to the FR2 region of IgE, a human IgE anti-sense TCL or comprehensive TCL library can be screen using the above FR2 target sequences.

An alternative way to generate human IgE blocking polypeptide which targets the FR2 region is to screen from a phage display human antibody library. In this case, each peptide encoded by the targeted FR2 sequence is synthesized artificially and used for panning as described below.

Example 6: Construction of a human IgE anti-sense TCL on bacteria phage

The human IgE anti-sense TCL is prepared by using coding sequences of the cDNA clones of human IgE light chain and heavy chain. The cDNAs of human IgE are separated from the vector sequences by agarose gel electrophoresis. Equal amounts of each purified inserts are mixed and used as templates for a random priming reaction.

~~Approximately 50 ng of the mixed template is boiled with 1 pmol of the~~ primer No. 1 (5'-GACGTGGCCN$_6$-3', N can be A, T, C, or G) for ~~3 min~~, cooled on ice, mixed with a reaction mixture containing 10 mM ~~Tris.~~HCl (pH 7.5), 10 mM MgCl2, 1 mM dithiothreitol, 0.125 mM each dNTP, and 2.5 units of Klenow fragment of DNA polymerase and incubated at 37 °C for 15 min. Then the reaction mixture is boiled and cooled again, another 2.5 units of Klenow fragment is added, and the reaction mixture is ~~incubated at 37 °C for 15 min.~~

~~The reaction is stopped by boiling and diluted 1:10 with TE buffer and~~ products are separated from primers using ~~Microcon-100~~ microconcentrators (Amicon) with two washes ~~of the~~ retained solution with 200 ml of the TE buffer. After two cycles of priming, some reaction products will incorporate primer sequences at both ends. They are

amplified in PCR using primer No. 2 (5'-GGCCGACGTGGCC-3') (SEQ ID NO. 16). The amplified products are precipitated, purified with Microcon-100, cut with sfiI and cloned into the sfiI site of the fUSE5 vector (71, 72). Amplification of the IgE-TCL follows the methods described by Smith and Scott (71), the details which are given in the manual supplied with the fUSE expression kit by Smith (72).

Primer No.1: 5'-GACGTGGCCTGTN6-3' (SEQ ID NO. 17) and primer No. 2: 5'-GGCCGACGTGGCCTGT-3' (SEQ ID NO. 18) are used to generate a constrained IgE-TCL.

Example 7: Selection of the IgE binding complementary anti-sense peptide by panning the human IgE TCL

Human IgE is diluted in PBS to a concentration of 20 mg/ml and is used to coat 3.5 cm wells by incubating for 1 h at 4 °C. The remaining binding sites are saturated by bovine serum albumin (BSA). A portion of the amplified IgE-TCL is first incubated for 2 h at 4 °C in a 3.5 cm well precoated with 1 mg/ml BSA in PBS and 1 mM MnCl2. The phage unbound to BSA are transferred to a similar well precoated with human IgE. After incubation for 1 h at 4 °C, the unbound phage are removed by washing 10 times with PBS buffer containing 0.5% Tween 20. The bound phage are eluted with 0.1 M glycine buffer, pH 2.2, containing 1 mg/ml BSA and 0.1 mg/ml phenol red.

The phages are amplified using the K91kan bacteria and partially purified by precipitation with polyethylene glycol (72). The panning is repeated for two more rounds. Sequences carried by the selected phage are then determined using the Sequenase kit (United States Biochemical) with the primer 5'-CCCTCATAGTTAAGCGTAACG-3' (73) (SEQ ID NO. 19).

Example 8: Characterizing anti-IgE activities of the complementary anti-sense peptides

The peptides selected from the previous step are synthesized and individually tested in the following assays.

Assay 1: Histamine release by passively sensitized basophils.

The procedure is described in detail previously (74). Briefly, reaginic sera are obtained from allergic patients who are allergic to ragweed, rye grass, Chinese elm or other preidentified allergens. Peripheral blood mononuclear cells are isolated from normal individuals by differential centrifugation on Ficoll-Pague (Pharmacia). Aliquots of a cell suspension are incubated with 2- to 10-fold dilution of a reaginic serum, Tris A buffer

containing 0.025 M Tris, 0.12 M NaCl, 0.005 M KCl, and 0.05% human serum albumin (pH 7.5) at 37 °C for 90 min.

The cells are then washed three times with Tris A buffer and resuspended in Tris-ACM buffer (pH 7.6) which consists of Tris A buffer plus 0.6 mM CaCl2 and 0.01 mM MgCl2. They are incubated with peptides at various concentrations for 30 min at 37 °C and then are challenged with either purified ragweed antigen E (0.1 mg/ml) or crude ragweed antigen, crude Chinese elm extracts, and rye grass extracts (Hollister-Stier, Spokane, WA).

After 40-min incubation at 37 °C, histamine content in supernatants is measured by the method of Siraganian (75). The peptides which can prevent histamine release triggered by all allergens will be selected.

Assay 2: Prausnitz-KDstner reactions

The procedure is carried out as described previously (74, 75). The reactions are done in a single healthy individual whose serum IgE concentration is at low level (around 10 ng/ml). To determine the abilities to block passive sensitization, 20 ml of various concentrations of the peptides is injected intracutaneously into skin sites 1 hr before sensitization of the skin sites with 20 ml of a 50-fold dilution of allergic serum (IgE at 912 ng/ml).

In each experiment, control site receives only the diluting buffer before the sensitization. All skin sites are challenged 48 hr after the passive sensitization with allergen extract, such as crude ragweed antigen. The size of wheat and erythema reactions are measured 20 min later by using transparent tape to transfer the outline of the reactions to a paper and expressed by the weight of the paper (76). It has been demonstrated that unsensitized skin sites do not show any wheat or flare reaction upon antigen challenge. Peptides which can prevent or lessen the reactions will be selected.

Example 9: Measuring affinity of the IgE-binding peptides and improvement of the peptides

The binding of peptides to human IgE can be analyzed by BIAcore biosensor (Pharmacia Biosensor AB, Uppsala, Sweden) (77, *see* 78 for review). Experiments are carried out at 25 °C in 10 mM HEPES buffered saline (HBS) with 150 mM NaCl, 3.4 mM EDTA and 0.005% Surfactant P20 (Pharmacia).

Human IgE is coupled to the sensor chip by activating the carboxymethylated dextran matrix with 0.2 M N-ethyl-N'-(3-diethylaminopropyl)-

carbodiimide and 0.05 M N-hydroxysuccinimide (NHS) (Pharmacia Amine Coupling Kit), followed by addition of the human IgE (80 mg/ml, 10 mM sodium acetate, pH 4.5). Remaining NHS-esters are blocked with a pulse of 1.0 M ethanolamine hydrochloride, pH 8.5.

To monitor the binding of peptides to IgE, various concentrations of peptide are injected over the immobilized IgE using conditions where binding is limited by kinetic parameters. Under these conditions, the solution association constant (KA) for the IgE-peptide interaction can be calculated using the following equation:

$$[SL]=KA[S]t[L]/(1+KA[L]) \quad (79)$$

where [SL] is the concentration of the IgE-peptide complex at equilibrium, [L] is the concentration of the free peptide, and [S]t is the total concentration of IgE.

Because the peptides selected in the present invention prevent the binding of allergens to IgE by steric hindrance rather than directly competing with the allergens for binding sites on IgE, peptides with apparent affinity comparable or even lower than the affinity between IgE and allergens can be effective in preventing the binding of allergens as long as enough concentration of the peptides is maintained locally so that they remain bound to IgE when the presensitized mast cells or basophils encounter the allergens. Nevertheless, if peptides with higher affinity are desired, an *in vitro* affinity maturation process can be performed using the TCLT-selected peptides as templates. The details of this approach are described in U.S. patent 5,223,409, incorporated by reference herein in its entirety.

The human IgE FR2 segment binding peptides can also be selected by constructing a cTCL against the IgE FR2 segment, and screening the cTCL with a synthetic peptide containing the IgE FR2 sequence. The screening procedure and characterization of the selected peptides are carried out as described above.

REFERENCES

1. Clackson, T. and Wells, J. A. (1994) In vitro selection from protein and peptide libraries. TIBTECH 12:173-184; Meeting report: Diversity by design. TIBECH 16:99-102, 1998

2. Koivunen, E., et al., (1993) Selection of peptides binding to the a5b1 integrin from phage display library. J Bio Chem 268:20205-20210

3. Cortese, R., et al., (1994) Epitope discovery using peptide libraries displayed on phage. TIBECH 12: 262 - 267

4. Cheng, X., et al., (1996) Identification of a biologically significant DNA-binding peptide motif by use of a random phage display library. Gene 171:1-8

5. D'Mello, F., et al., (1997) Definition of the primary structure of hepatitis B virus (HBV) pre-S hepatocyte binding domain using random peptide libraries. Virology 237:319-326

6. Birkenmeier, G., et al., (1997) Epitope mapping by screening of phage display libraries of a monoclonal antibody directed against the receptor binding domain of human a2-macroglobulin. FEBS Letters 416:193-196

7. Sxardenings, M., et al., (1997) Phage display selection on whole cells yields a peptide specific for melanocortin receptor 1. J Bio Chem 272:27943-27948

8. Fack, F., et al (1997) Epitope mapping by phage display: random versus gene-fragment libraries. J Immunol Methods 206:43-52

9. Kiewitz, A. and Wolfes, H. (1997) Mapping of protein-protein interactions between c-myb and its coactivator CBP by a new phage display technique. FEBS Letters 415:258-262

10. Nord, K., et al., (1997) Binding proteins selected from combinatorial libraries of an a-helical bacterial receptor domain. Nature Biotech 15:772-777

11. Blalock, J. E. and Smith, E. M. (1984) Hydropathic anti-complementarity of amino acids based on the genetic code. Biochem. Biophys Res. Commun. 121:203-207

12. Bost, K. L., et al., (1985) Proc. Natl Acad. Sci. USA 82:1372-1375

13. Blalock, J. E. and Bost, K. L. (1986) Biochem. J. 234:679-683

14. Clarke, B. L. and Blalock, J. E. (1990) Steroidogenic activity of a peptide specified by the reversed sequence of corticotropin mRNA. Proc. Natl. Acad Sci USA 87:9708-9711

15. Baranyi, L., et al., (1995) The anti-sense homology box: a new motif within proteins that encodes biologically active peptides. Nature Med 9:894-901

16. Fassina, G., et al., (1992) Design of hydropathically complementary peptides for human big endothelin affinity purification. Int J Peptide Prot Res 39:540-548

17. Lu, F. X., et al., (1991) Affinity capture of [Arg8] vasopressin-receptor complex using immobilized anti-sense peptide. Proc Natl Acad Sci USA 88:3642-3646

18. Fassina, G., et al., (1992) Synthesis of a peptide ligand for bradkinin affinity purification. in Peptides 92 (eds Schneider, C. H. & Eberle, A. N.) 22nd Eur Peptide Soc Meeting

19. Scapol, L., et al., (1992) Purification of recombinant human interferon beta by immobilized anti-sense peptide strategy. J Chromatogr 600:235-242

20. Bost, K. L., et al. (1985) Similarity between the corticotropin (ACTH) receptor and a peptide encoded by an RNA that is complementary to ACTH mRNA. Proc Natl Acad Sci USA 82:1372-1375

21. Bost K. L., et al (1985) Regions of complementarity between the messenger RNAs for epidermal growth factor, transferrin, interleukin-2 and their respective receptors. Biochem Biophys Res Commun 128:1373-1380

22. Kelly, J. M. et al. (1990) Vasopressin anti-sense peptide interactions with the V1 receptor. Peptides 11:857-862

23. Ruiz-Opazo, N. et al. (1995) Identification of a novel dual angiotensin II/vasopressin receptor on the basis of molecular recognition theory. Nature Med 1:1074-1081

24. Soffer, F. L. et al. (1987) Binding of a novel peptide to the angiotensin II receptor. Proc Natl Acad Sci USA 84:9219-9222

25. Moore, G. J. et al. (1989) Angiotensin antipeptides: a (-)messenger RNA complementary to human angiotensin II (+) messenger RNA encodes an angiotensin receptor antagonist. Biochem Biophs Res Commun 160:1387-1391

26. Shahabi, N. A. et al. (1992) Characterization of antisera to the naloxon insensitive receptor for beta endorphin on U937 cells generated by using the complementary peptide strategy. J Pharmac Exp Ther 263:876-883

27. Fassina, G. et al. (1989) Recognition properties of peptides hydropathically complementary to residues 356-375 of the c-Raf protein. J Biol Chem 264:11252-11257

28. Carr, D. J. J. et al (1989) Monoclonal antibody against a peptide specified by [Met]-enkephalin complementary RNA recognizes the delta-class opioid receptor. Immun Lett 20:181-186

29. Pasqualini, R. et al. (1989) Determination of the putative binding site for fibronectin on platelet glycoprotein IIb-IIa complex through a hydropathic complementarity approach. J Biol Chem. 264:14566-14570

30. Brentani, R.R. et al (1988) Characterization of the cellular receptor of fibronectin through a hydropathic complementary approach. Proc Natl Acad Sci USA 85:364-367

31. Carr, D. J. J. et al (1986) An antibody to a peptide specified by an RNA that is complementary to gamma-endorphin RNA recognizes an opiate receptor. J Neuroimmun 12:329-337

32. Mulchahey, J. J. et al (1986) Antibodies to the binding site of the receptor for leuteinizing hormone-releasing hormone (LHRH): Generation with synthetic decapeptide encoded by an RNA complementary to LHRH mRNA. Proc Natl Acad Sci USA 83:9714

33. Campbell-Thompson, M. and McGuigan, J. E. (1993) Canine parietal cell binding by antibodies to the complementary peptide of somatostatin. Am J Med Sci 305:365-373

34. Bret-Dibat, J. L. et al (1994) Antipeptide antibodies that recognize a substance P-binding site in mammalian tissues: a biochemical and immunochemical study. J Neurochem 63:333-343

35. Borovsky, D. et al. (1994) Characterization and localization of mosquito-gut receptors for trypsin modulating oostatic factor using a complementary peptide and immunocytochemistry. FASEB J. 8:350-355

36. Martins, V. R., et al., (1997) Complementary hydropathy identifies a cellular prion protein receptor. Nature Med 12:1376-1382

37. Elton, T. S. et al (1988) Purification of an angiotensin II binding protein by using antibodies to a peptide encoded by angiotensin II complementary RNA. Proc Natl Acad Sci USA 85:2518-2522

38. Blalock, J. E. (1990) Complementarity of peptides specified by 'sense' and 'anti-sense' strands of DNA. TIBTECH 8:140-144

39. Brentani, R. R. (1988) Biological Implications of complementary hydropathy of amino acids. J. Theor. Biol. 135:495-49

40. Brentani, R. R. (1990) Complementary hydropathy and the evolution of interacting polypeptides. J Mol Evol 31:239-243

41. Segersteen, U., et al., (1986) Frequent occurrence of short complementary sequences in nucleic acids. Biochem Biophy Res Commun 139:94-10

42. Johnson, H. M. & Torres, B. A. (1988) A novel arginin vasopressin-binding peptide that blocks arginin vasopressin modulation of immune function. J Immun 141:2420-2423

43. Zamai, M. & Caiolfa, V. R. (1994) Sequence-directed recognition peptides: inhibition of endothelin generation via a substrate depletion mechanism. Biochem Biophys Acta 1202:337-340

44. Dillon, J. D. et al. (1991) A peptide mimeric of calcium. Proc Natl Acad Sci USA 88:9726-9729

45. Ghiso, J. et al. (1990) Binding of cystatin C to C4: the importance of sense-anti-sense peptides in their interaction. Proc Natl Acad Sci USA 87:1288-1291

46. Nagy, F. M. & Frawley, L. S. (1991) A peptide complementary to a predicted binding region of rat pituitary D2 receptor acts as a dopamine antagonist. 73rd Annu. Meeting, Endocrine Society, Abst. 716, p.209 (Endocrine Soc., Bethesda, Maryland).

47. Gartner, K. et al. (1991) The peptides APLHK, EHIPA, GSPL, are hydropathically equivalent peptide mimics of a fibrinogen binding domain of glycoprotein IIb/IIa. Biochem Res Commun 180:1446-1452

48. McGuigan, J. E. & Campbell-Thompson, M. (1992) Complementary peptide to the carboxyl-terminal tetrapeptide of gastrin. Gastroenterology 103:749-758

49. Bost, K. L. & Blalock, J. E. (1989) Production of anti-idiotypic antibodies by immunization with a pair of complementary peptides. J Molec Recognit 1:179-183

50. Whitacker, J. N. et al. (1989) Monoclonal idiotypic and antiidiotypic antibodies produced by immunization with peptides specified by a region of human myelin basic protein mRNA and its complement. J Neuroimmunol 22:157-166

51.Blalock, J. E. et al. (1989) Use of peptides encoded by complementary RNA for generating anti-idiotypic antibodies of predefined specificity. Methods in Enzymology 178:63-74

52. Kang, Ch-Y. et al. (1988) Inhibition of self-binding antibodies (autoantibodies) by Vh-derived peptide. Science 240:1-34-1036

53. Knutson, V. P. (1988) Insulin binding peptide. J Biol Chem 263:14146-14151

54. Fassina, G. & Cassini, G. (1992) Design and recognition properties of a hydropathically complementary peptide to human interleukin 1 beta. Biochem J 282:773-779

55. Castronovo, V. et al. (1991) Laminin receptor complementary-DNA deduced synthetic peptide inhibits cancer cell attachment to endothelium. Cancer Res 51:5672-5678

56. Araga, S. et al. (1993) Prevention of experimental myasthenia gravis by manipulation of the immune network with a complementary peptide for the acetylcholine receptor. Proc Natl Acad Sci USA 90:8747-8751

57. Pestov, D. G. & Lau, L. F. (1994) Genetic selection of growth-inhibitory sequences in mammalian cells. Proc Natl Acad Sci USA 91:12549-12553

58. Lupas, A. et al. (1991) Predicting coiled coils from protein sequences. Science 252:1162-1164

61.Ladner, R. C. (1995) Constrained peptides as binding entities. TIBTECH 13:

62. Kieber-Emmons, T. & Kohler, H. (1986) Towards a unified theory of immunoglobulin structure-function relations. Immun Rev 90:29-48

63. Greve JM, Davis G, Meyer AM, Forte CP, Yost ST, Marlor CW, Kamarck ME and McClelland A (1989) Cell 56:839-847

64. Hofer F, Gruenberger M, Kowalski H, Machat H, Huettinger M, Kuechler E and Blaas D (1994) Members of the low density lipoprotein receptor family mediate cell entry of a minor-group common cold virus. Proc Natl Acad Sci USA 91:1839-1842

65. Ishizaka, K. (1985) Immunoglobulin E (IgE). Method Enzymology 116:76-94

66. Naclerio, R. and Solomon, W. (1997) Rhinitis and inhalant allergens. JAMA 278:1842-1848

67. Ishizaka, T. et al. (1985) J Immunol. 134:532-540

68. Hakimi, J. et al. (1990) The a subunit of human IgE receptor (FcERI) is sufficient for high affinity IgE binding. J Bio Chem 265:22079-22081

69. Chatterjee, S. K. et al. (1998) Molecular mimicry of carcinoembryonic antigen by peptides derived from the structure of an anti-idiotype antibody. Cancer Res 58:1217-1224

70. Maier, C. C. et al. (1994) Identification of interactive determinants on idiotypic-anti-idiotypic antibodies through comparison of hydropathic profiles. Immunomethods 5:107-113

71. Smith, G. P. & Scott, J. K. (1993) Libraries of peptide and proteins displayed on filamentous phage. Methods Enzymol 217:228-257

72. Smith, G. P. (1992) Cloning in fUSE vectors. Feb 10, 1992 ed. Division of Biological Sciences, University of Missouri, CO.

73. Koivunen, E. et al. (1993) Selection of peptides binding to the a5b1 integrin from phage display library. J Bio Chem 268:20205-20210

74. Helm, B. et al. (1989) Blocking of passive sensitization of human mast cells and basophil granulocytes with IgE antibodies by a recombinant human e-chain fragment of 76 amino acids. Proc Natl Acad Sci USA 86:9465-9469

75. Siraganian, R. P. (1974) Anal. Biochem 57:534-540

76. Sussman, G. L. et al. (1982) Ann. Allergy 48:75-77

77. McDonnell, J. M. et al. (1996) Structure based design and characterization of peptides that inhibit IgE binding to its high-affinity receptor. Nature structural Biology 3:419-426

78. Jonsson, U. et al. (1991) Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. BioTechniques 11:620-627

79. Navia, M. A. & Murcko, M. A. (1992) use of structural information in drug design. Curr. Opin Struct Biol 2:202-210

80. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M., Gottesman, K. S. and Foeller, C. (1991).Sequences of Proteins of Immunological Interest, 5th ed. US Department of Health and Human Services, Public Service, NIH, Washington

81. Barbas III, C. F. (1995) Synthetic human antibodies. Nature Medicine 1:837-839

82. Greve, J. M. et al (1989) The major human rhinovirus receptor is ICAM-1. Cell 56:839-847

83, Staunton, D. E. et al. (1988) Primary structure of intercellular adhesion molecule 1 (ICAM-1) demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925-933

84. Staunton, D. E. et al. (1990) The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. Cell 61:243-254

85. McClelland, A. et al (1991) Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of intercellular adhesion molecule 1. Proc. Natl. Acad. Sci USA 88:7993-7997

86, Lineberger, D. W. et al (1990) Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor. J. Virol 64:2582-2587

87. Olson, N. H. et al (1993) Structure of a human rhinovirus complexed with its receptor molecule. Proc Natl Acad Sci USA 90:507-511

88. Greve, J. M. et al (1991) J. Virol 65:6015-6023

89. Scott , J. K. and Smith, G. P. (1990) Searching for peptide ligands with an epitope library. Science 249:386-390

90. M. Caruthers (1991) Accounts Chem Res 24:278

91. Kamtekar, S. et al., (1993) Protein design by binary patterning of polar and nonpolar amino acids. Science 262:1680-1685

92. Snow, R. E. et al., (1995) Analysis of Ig V_H region genes encoding IgE antibodies in splenic B lymphocytes of a patient with asthma. J Immunol 154:5576-5581

93. Wang, Y. and Yeh, M. (1996) Molecular characterization of the V regions of four IgE antibodies specific for trichosanthin. Immunology 89:316-323. All publications cited in the specification are incorporated by reference herein, including drawings and sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above. Other embodiments of this invention are disclosed in the following claims.